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Antibacterial Activity Test to Ethanol Extract of White Pumpkin Leaf (Lagenaria siceraria) Against Multiple Bacterial Pathogens

Gemy Nastity Handayany
Pharmacy Department, Universitas Islam Negeri Alauddin
Makassar, INDONESIA

Abstract
Research had done on the antibacterial activity of white pumpkin leaf extract (Lagenaria Siceraria) Against Multiple Bacterial Pathogens. This study aims to determine the antibacterial activity of ethanol extract of white pumpkin leaf to 8 pathogenic bacteria (Escherichia coli, Vibrio sp, Streptococcus mutans, Stapylacoccus aureus, Salmonella thypi, Pseudomonas aeroginosa, Bacillus subtilis and Staphylococcus epidermidis.) The study was conducted by extracting white pumpkin leaf by using ethanol 96%. Furthermore, the screening test conducted using microbes to extract ethanol from white pumpkin leaf by using a concentration of 0.5% and 1% (w / v) with a negative control piper disk. Result obtained is at a concentration of 0.5% can inhibit the four bacterial pathogens including Escherichia coli, Streptococcus mutans, Stapylacoccus aureus and epidermidis Staphylacoccus. After the preliminary test and then test the minimum inhibitory concentration (MIC) and the minimum kill concentration test (MKC), where the result obtained is at a concentration of 0.5% white pumpkin leaf extract could inhibit bacterial pathogens 4 obtained in the screening test and a concentration of 2 % white pumpkin leaf extract capable of killing four bacterial pathogens.Further testing is testing the inhibition by diffusion method against some pathogenic bacteria that inhibit the screening result (Escherichia coli, Streptococcus mutans, Stapylacoccus aureus and epidermidis Staphylacoccus) with a concentration of 0.5%, 1% and 2% where chloramphenicol as a positive control. Results obtained diameter zone of the biggest barriers to the bacteria Escherichia coli, Streptococcus mutans, Stapylacoccus aureus and epidermidis Staphylacoccus row was 2.2 cm; 4.1 cm; 2.43 cm and 3.73 cm.
Keywords: Ethanol extract, White pumpkin leaf, Pathogenic bacteria

1. Introduction

White pumpkin is a plant that is relatively easy to grow because it is able to adapt to the environment both in the highlands and lowlands. In addition, this plant is able to adapt to the lack of water in the dry season and the excess water during the rainy season. This plant is cultivated by seeds (amit Kumar, 2012).

White pumpkin (Lagenaria siceraria) contains 95% water, 3.5 to 6.3% carbohydrate, 1.5% fiber, 0.5-0.7% protein, o, 1 to 0.2% fat, calcium , phosphorus, provitamin A, vitamin B1, vitamin B2, vitamin B3, vitamin C and contains saponin and polyphenols (Widyaningrun, 2011). On white pumpkin leaves (Lagenaria siceraria) are saponins (Lagenaria siceraria) are estimated to have antiseptic (amit Kumar, 2012).

Empirically juice of white pumpkin flesh (Lagenaria siceraria) used to treat high fever as a result of typhus or infection as much as half a glass, taken twice a day in the morning and afternoon (Widyaningrum, 2011). Fruit, leaves, oil and roots are used in traditional medicine as an anthelmintic, diabetes, hypertension, skin rashes, and diuretics addition of white pumpkin leaf (Lagenaria siceraria) is used as an insomnia drug (Rakesh Prajapati P. 2010). One of the things that cause disease in humans is due to the presence of bacteria, wherein the bacteria or microbes are living organisms that are so small and can only be seen using a microscope. The bacteria can enter the digestive tract via the food, beverage and through contaminated fingers (Umar, 2004).

Pathogenic bacteria are one type of harmful bacteria and cause a variety of diseases, both in the human body, animals and plants. Some pathogenic bacteria that can cause illness include Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Salmonella thypi, Staphylacoccus aureus, Staphylacoccus epidermidi, Streptococcus mutans and Vibrio sp. Therefore, these bacterias used in this study. As for the content of white pumpkin leaves are saponins which have antiseptic or able to inhibit the growth of bacteria. So the use of white pumpkin leaves can be used to inhibit bacterial activity, which empirically white pumpkin leaves are used as a traditional medicine as an anthelmintic, diabetes, hpertensi, itchy skin, diuretic and helps digestion in the body.

Saponin in the white pumpkin leaf is one of the glycosides that are found in plants. Saponin is characterized by froth. So when treated with water and whipped it will form a froth which can last a long time. Saponin is easily soluble in water and insoluble in ether. So, ethanol is used as a solvent to attract saponins contained in white pumpkin leaf, where ethanol is one of the polar compounds or compound easily soluble in water (Lei et al., 2002). Based on the above figures do this research by testing the antibacterial activity of ethanol extract of white pumpkin leaves (Lagenaria siceraria) against several bacterial pathogens.

2. Questions Research

1. How does the antibacterial activity of ethanol extract of white pumpkin leaf (Lagenaria Siceraria) against

several pathogens?
2. At what concentration of antibacterial activity of ethanol extract of white pumpkin leaf (Lagenaria Siceraria) can inhibit pathogenic bacteria?

3. Plant Description

White pumpkin empirically been used by people to treat various diseases such as typhus. White pumpkins have the name of various different countries including: Bengali: Lau, Chinese: Hu, Hu Lu Gua, Hulu, Hu Gua, Gua Mao, Peh Poh. Danish: Flaskegræskar, Flaskegraeskar, Kalabas. Dutch: Fleskalebas, Flessepompoen. Finnish: Pullokurpitsa. French: Pumpkine Bouteille, Coupumpkine, Calebassier, Calebasse. Finnish: Pullokurpitsa. German: Flaschenkürbis, Flaschen-Kurbis, Trompetenkürbis, Kalebassenkürbis. Hindi: Dudhi (Dudi, Dodi), Lokhi (Lauki). Indonesian: Pumpkin Bottle, Water Pumpkin, Pumpkin White Italian: Zucca Da Tabacco, Zucca Da Vino. Japanese: Yuugao. Khmer: Khlook .. Laotian: Namz taux .. Malayalam: Sorekai .. Marathi: Charanga.Nepalese: Laukaa, Tito Tumba. Oriya: Lau.. Portuguese: Abóbora-Carneira, Cabaço. Punjabi: Dudhi. Sinhalese: Diya Labu. Spanish: Calabaza Vinatera, Cogorda, Cajombre, Calabaza, güiro Amargo. Swedish: Kalebass, Flaskkurbits. Telugu: Beerakaya. Thai: Namtao (Naam Tao), Manamtao, Khi Luu Saa.

In Indonesia Pumpkins have several different names, namely Pumpkin water (Sumatra), Pumpkin Frangi (Malay), Tabu (North Sumatra), Kukuk (Sunda), Pumpkin ayer, Waluh Kenti (Java), Pumpkin lente (Madura), Karobu (East Sumba) (Shah, 2010). While white pumpkins have different kinds of regional names such as: Bulukumba; kunrulu, Jeneponto: boyo china; Gowa; maradduse; Java: white pumpkin and Jakarta: Pumpkin water or white pumpkins (Kubde, 2010).

3.1 Morphology

Pumpkin is an annual herbaceous plant that grows spread, has a square-shaped stem, with pembelit tool. Single-stemmed leaves are cylindrical, rough surfaces and green. Flowers monoecious in the axillary leaf, yellow-green, have five crowns, five stamens and pistils 3. Fruit elongated round and yellowish green, with hard textured skin. It has many fruit seeds, flattened, oval and white and tap rooted. Pumpkin is a plant that is relatively easy to grow because it is able to adapt to the environment both in the highlands which have low temperature and lowlands have high temperature.

In addition, this plant is able to adapt to the lack of water in the dry season and the excess water in the rainy season. This plant is grown from seed. The grain needs 4-5 seeds / ha, with ground-breaking 2 times a day in order to loose and manure. With the harvest period is 70-90 days is quite fast depending on the desired level of development of the fruit. At the time of harvest pumpkins stalks should be cut with a knife but do not fall. In cutting, leaving the fruit stalk about 5 cm, so do not cut intact (Shah, 2010).

3.2 Chemical Ingredients

Chemical constituents of white pumpkins are nutritional values per 100 grams of raw white pumpkin (3.5 oz), Energy 10 kcal 60 kJ, 6.5 g carbohydrates, sugar 1.36 g, dietary fiber 0.5 g, fat 0, 1 g, 0.05 g saturated fat, monounsaturated fats 0.01 g, polyunsaturated fats 0.01 g, 1 g protein, Vitamin A 369 mg (41%), β-carotene 3100 mg (29%), Thiamin (Vit. B1) 0.05 mg (4%), Riboflavin (Vit. B2) 0.110 mg (7%), Niacin (Vitamin B3.) 0.6 mg (4%), Pantothenic acid (B5) 0.298 mg (6%), 0.061 mg Vitamin B6 (5%), Folate (Vit. B9) 16 mg (4%), Vitamin C 9 mg (15%), Vitamin E 1.06 mg (7%), Calcium 21 mg (2%), iron 0.8 mg (6%), Magnesium 12 mg (3%), phosphorus 44 mg (6%), Potassium 340 mg (7%), Sodium 1 mg, 0.32 mg Zinc (3%), saponin, Polyphenols (amit Kumar, 2012).

Table 1. Nutrional components of Lagenaria Siceraria: (per 100g of fruit section)

Nutrient components	Denomination	Value
Water moisture	G	96,1
Protein	G	0,2
Fat	G	1,0
Mineral	G	0,5
Fiber	G	0,6
Carbohydrates	G	2,5
Energy	Calorie	12
Calcium	Mg	120
Magnesium	Mg	5
Phosphorous	Mg	10
Iron	Mg	0,7
Sodium	Mg	1,8
Potassium	Mg	87
Copper	Mg	0,3
Sulphur	Mg	10
Vitamin-A	IU	60
Thiamine	Mg	0,03
Riboflavin	Mg	0,01
Nicotinic acid	Mg	0,2
Vitamin-C	Mg	5
Oxalic acid	Mg	27

(Source: Kumar Amit, 2012)

While the white pumpkin leaf (Lagenaria Siceraria) containing cucurbitacin B. Where, cuucurbitasin B contains saponin while oil is obtained from the leaf clear and yellow pale. Seeds provide 45% of the oil with the following characteristics: iodine value, 126.5; free fatty acids, 0.54%; and materials are saponified, 0.67%. Components of free fatty acids are: linoleic acid, 64.0; Oleic, 18.2; and saturated fatty acid, 17.8%.

The content of white pumpkin leaf is saponin, wherein the saponin is of secondary metabolites, which are able to form foam, and can haemolysis red blood cells. The formation of foam when extracting crude drug is evidence of saponins (Harbone in wawolumaya, 2012). Saponins have the characteristics of foam. When treated with water and whipped it will form foam, which could last a long time. Saponin is easily soluble in water and insoluble in ether. Saponins have a bitter taste piercing and cause sneezing and irritation of the mucous membranes. Saponin is a poison that can destroy blood grains or hemolysis in blood. Saponins are toxic to the cold-blooded animals and are widely used as poison on fish. Saponins are hard. Saponins an antibacterial compound is a biological or chemical compound that can kill or inhibit the growth of antibacterial activity by inhibiting protein synthesis or binding of ribosomes (Hartono in Wawolumaya, 2011).

4. Plant Efficacy

Many people favor to pumpkin because of the benefits. Besides containing minerals, water, calcium, iron, and vitamin C, also contains saponins and polyphenols. Generally, within 100 flasks are containing 1.1 g protein, 0.3 fat, mineral 0.8, and 45 mg of calcium. On the leaves and fruit contain saponins and polyphenols. Has not been much research on white pumpkin, white pumpkin but it is believed to have efficacy can lower the body heat. Based on the research results Herman in "The Effect of Infusion of fruit Benincasa hispida, Corn, with Infusion fruit Lagenaria leucantha, Rusby to white rat's body temperature" showed that the both fruits have the same effect in reducing fever. Alternative medicine expert Hembing also revealed that the pumpkin has efficacy for treating high blood pressure, lower the heat, diabetes, and facilitate the digestive process. Polyphenols and saponins is one fito chemicals that have the effect of inhibiting the growth of cancer biology, antioxidants, inhibit microbial growth, lower blood cholesterol, decrease blood levels, are antibiotics and can boost immunity (Kubde, 2010).

White color in white pumpkin hinted the abundance of mineral deposits that serves to control nerve health (among others as a stress reliever), reduced the risk of cancer and suppress the growth of cancer cells, debilitating viruses and bacteria that can be used to solve the health problems caused by viruses or bacteria, such as flu, vaginal discharge and inflammation (Kubde, 2010). Pumpkins are cold and wet which gives mild nutritional intake. Its soft and watery foods can give injections damp, slimy, and very well suited for those who are cold and excess mucus (Kubde, 2010).

Flesh, leaves, oil, and seeds are edible and are used by local people as a folk remedy in the treatment of jaundice, diabetes, ulcers, hemorrhoids, colitis, madness, hypertension, congestive heart failure, and skin

diseases. Flesh is used as vomiting, sedative, laxative, refrigerant, diuretic, antibilious, and chest. The flowers are the antidote. The bark and the rind are used as a diuretic while the seed is used as an anthelmintic. The plant extracts have shown antibiotic activity. The water decoction of white pumpkin leaves are widely used for baldness (Rakesh Prajapati P, 2010).

The water can quench the thirst and very nutritious. If it is taken as many as 15 times in the form of jam, it can dissolve mucus. If crushed and then wrapped on top of the head can help overcome inflammation of the brain. The juice of flesh when mixed with rose water, then dripped into the ear, the ear swelling merit overcome. Pumpkin is also nutritious treat eye inflammation and gout heat. In addition, it can control blood sugar levels. For those who have digestive heat and has a fever, it is recommended to consume this fruit (Kubde, 2010).

The content number of rare amino acids is efficacious to prevent or treat benign prostatic hypertrophy or enlargement in older men. On the red pumpkin seeds contain minerals Zn (zinc) and Mg (Magnesium), which is very important for the health of the reproductive organs, including the prostate gland. Scientists from Chosun University, South Korea found that the skin of the pumpkin there is a kind of active substance that can kill disease-causing germs Candidiasis or fungal infection (Kubde, 2010) .

Ribosome prokaryotic cells have a molecular mass of 2.52 million dalton and its dimensions is 29 X 21 nanometer. Ribosomes cells - eukaryotic cells is greater than the cell ribosome - the cell prokaryotes. Molecular mass ribosomal eukaryotic cells ranged from 4.22 million dalton and matranya 32 X 22 nanometer. Size - the size is determined by the analysis of ribosome sedimentation (precipitation). This analysis is based on measuring the rate of deposition of a molecule or particle in viscous solution, usually sucrose solution which at very high speeds (70g or more). Konfesiensi sedimentation expressed in S is unity or unit Swedberg. In addition Swedberg coefficient, the rate of deposition is also influenced by factors - another factor is the molecular weight, weight macromolecule, or macro-molecular assemblies. Ribosome prokaryotic has a sedimentation coefficient of 70S, whereas in eukaryotic cell sedimentation coefficient of 80S. Ribosomes cell prokaryotes, when in solution with low levels of Mg ++ for example 0.2 mm will experience dissociated into two sub-units of different sizes or sedimentation coefficient. Large sub-unit 50S has a sedimentation coefficient, while the sedimentation coefficient 30S small.

Since all proteins and ribosomal RNA sub-unit prokaryote can be in isolation, which enables it to explain the process of arranging the ribosome through the study of recombination. This shows that the preparation of the sub-units and merging to form functional ribosomes (capable of translating mRNA into protein) that occurred when spontaneously the invitro rRNA and protein components can be used. The preparation can be done by itself and its complement structure of protein molecules and ribosomal RNA that is processed through the formation of hydrogen bonds and hydrophobic interactions.

The addition of certain proteins in the formation of sub-units can be easy addition and other binding. When the L protein is added to the 16S RNA or protein S when added to the 5S and 23S RNA, then there will be drafting. RNA 20S sub-unit of one species can join the S protein of prokaryotes others will form functional subunits, also for protein and 50S RNA from different prokaryotes. Preparation of sub-units and the formation of a hybrid functional monomer proved difficult because the proteins and ribosomes and RNA from different prokaryotes in fact have a different primary structure. So it is clear that the secondary and tertiary structures are very similar rRNA is more important in the interaction of proteins, so although some ribososme protein from yeast cells, retikolosit, and liver of mice can be replaced by protein from E. coli, but the hybrid formed from the monomer sub-unit prokaryote and eukaryotes will not function in protein synthesis.

Ribosome prokaryotic containing RNA and protein. In ribosome prokaryotic sub-unit contains an RNA molecule that is the RNA 16S (BM 0.6 X 10^6) while the large sub-unit of RNA molecules that contain two 23S RNA (BM 1.6 X 10^6) and 5S RNA (BM 3.2 X 10^4). Third RNA is the transcription product in a closely chain genes in order of 16 S - 23 S - 5 S. Small sub-unit protein has a molecular weight of between 10,900 (S17) to 65,000 (S1), whereas the protein sub-units between 9600 (L34) to 31500 (L2). Ribosomal protein is generally wet, soggy rich in amino acids and has an isoelectric point at pH 10 or more, about 33 of 55 proteins has been sorted (13 of sub-unit 20 of the small and large sub-units). Along with the research on RNA, the assumption is the prokaryotic ribosome is an organelle that is well understood in terms of structure and function (Reksoatmodjo, 1993).

Antimicrobials affect nucleic acid metabolism. For example rifampicin binds and inhibits DNA-dependent RNA polymerase that exists in bacteria. Quinolones inhibit DNA gyrase, and metronidazole inhibits DNA synthesis (Djide, 2008).

The test method is frequently used antimicrobial diffusion jelly plate method. This test is performed on the surface of a solid medium. Determining the activity of antimicrobial agents, the dish is containing antimicrobial placed on the jelly media. Microbe was grown on the surface of the medium and the disk-shaped filter paper that had been containing microbes. After incubation the inhibition zone diameters were measured. Area clearly indicates the existence of inhibition of microorganisms by an antimicrobial agent on the surface of a

jelly media. Diameter of inhibition zone is a measure indirectly MIC of antibiotics against microbes. Clinical sensitivity of microbes is then determined from the classification table (Greenwood, 1995).

Table 2. Classification responses to bacterial growth inhibition

Light Zone Diameter	Inhibition Growth Responses
>20 mm	Strong
16-20 mm	Medium
10-15 mm	Weak
<>	None

Source: Greenwood, 1995

Antibacterial and antimicrobial test method is another technique of Dilution Test Tube. Its function is to determine the results of MIC directly. Another method is the E-test method, which is a jelly diffusion test method that is easily and quickly obtain the results of MIC (Underwood, 1995). Several factors can affect the size of the zone of inhibition and should be controlled are: (a) The concentration of microbes on the medium surface. The higher the concentration of microbial inhibition zones will be smaller; (B) The depth of the medium petri dish. The thicker the medium in a petri dish, the inhibition zones will be smaller; (C) The pH value of the medium. Some antibiotics work well on some acidic alkaline conditions, Aerobic conditions or anaeorob. Some antibacterial its best work on aerobic conditions and the other in aerobic conditions (Greenwood, 1995).

5. Research Methods

This research is a qualitative research that is experimental laboratory. The samples used in this study were white pumpkin leaves (Lagenaria siceraria). The tools used autoclave (Hirayama®), brown bottle, a petri dish (Iwake Pyrex®), 250 ml glass Erlenmeyer (Iwake Pyrex®), 250 ml beaker (Iwake Pyrex®), a measuring cup 100 ml (Iwake Pyrex®), incubator (Memmert®), calipers, gas stove (Rinnai®), Laminar Air Flow (LAF) (Esco®), light spirits, ose round, oven (Memmert®), a water bath, tweezers, rotary evaporator (IKA®) , tube racks, spoit (One Med®), test tubes (Iwake Pyrex®), an analytical balance (AND®), and the vial.

Materials used: Distilled, aluminum foil, white pumpkin leaves (Lagenaria siceraria), DMSO (dimethyl sulfoxide), cotton, paper weigh, pure bacterial culture (Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus, Stahylococcus epidermidis , Streptococcus mutas, Vibrio sp), medium Jelly Nutrient , medium Glucose Jelly Nutrient , medium Nutrient Glucose Borth (GNB), 0.9% NaCl, ethanol, paper disc.

5.1 Work procedures

Processing of samples: Samples of white pumpkin leaves (Lagenaria siceraria), which have been obtained are cleaned by using running water and then drained. White pumpkin leaves that have been cleaned and sorted and weighed wet. White pumpkin leaves then thinly sliced and then dried for several days. Simplicia which has been dried and then stored in a sealed plastic container.

Sample extraction: white pumpkin leaves (Lagenaria siceraria) weighed 200 g, and then put into the jar. Sufficiently, it wetted with ethanol then soaked with ethanol until the entire sample is submerged (25000 ml of ethanol) and allowed to stand for 1 x 24 hours while stirring occasionally. It is separated between the filtrate and dregs while pulp macerated again by using ethanol for 1 x 24 hours then the results are filtered with filter paper and aerated to obtain a white pumpkin leaf extract (Lagenaria siceraria) free of ethanol. The extract obtained is then measured in volume using a measuring cup and then put in a brown glass bottle and sealed.

Equipment sterilization: Glassware such as petri dishes and other tools used to breed microorganisms should be sterilized in an autoclave at a pressure of 2 atm at a temperature of 1200C for 15 minutes. For pipette which has been used to take the microorganisms should be disinfected with a solution of phenol 5%. Then glassware was washed with hot detergent for 15-30 minutes followed by rinsing first with 0.1% HCl and finally with distilled water. The tools are dried upside down in the open air after the dry pack with parchment paper. Then before sterilized, the petri dish is wrapped first with paper while the jars, vials and glass Erlenmeyer advance corked with cotton net. The glass tools were in an oven at a temperature of 1800C for 2 hours. Syringe and a plastic tool (not high-heat resistant) sterilized in an autoclave temperature of 1210C for 15 minutes with a pressure of 2 atm. Ose needle is sterilized by direct heating to anneal.

Preparation of Bacteria Test: Bacteria samples used in this study was Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus, Stahylococcus epidermidis, Streptococcus mutas, Vibrio sp. Bacteria from pure cultures were rejuvenated in the medium Jelly Nutriet (NA) slant and incubated for 1 x 24 hours at a temperature of 370C.

The Making Suspension Bacteria Test: Bacteria test was 24 hours were suspended in 10 ml physiological saline solution (0.9% NaCl) was then measured absorbance in 25% of T with a UV-VIS spectrophotometer at a wavelength of 580 nm.

Antibacterial Screening Tests: A total of 50 mg of ethanol extract of white pumpkin leaves (Lagenaria siceraria) each dissolved in 0.2 mL of DMSO by using a micropipette, then mix with 9.8 ml of medium NA to obtain a final volume of 10 ml. the mixture was poured into a petri dish aseptically to shake so evenly and allowed to solidify. Piper bacterial cultures were then inserted the disk that had been poured 20 µ samples that had been diluted with a concentration of 0.5%, 1% and 2% over the medium and incubated at a temperature of 370C for 1x24 hours. No activity was observed in the absence of microbial growth medium.

Antibacterial Testing:

a. **Testing MIC (Minimum Inhibitory Concentration)**: MIC Testing conducted by making 3 dilution of the ethanol extract of white pumpkin leaves (Lagenaria siceraria) of 0.5%; 1%; and 2%. Created a stock solution of ethanol extract of leaves of white pumpkin (Lagenaria siceraria) 2%, and diluted with 0.2 ml DMSO, then added to 10 ml of medium NAM. The volume of each tube dilution of stock solution are taken in accordance with the calculation and adequated with GNB medium, then add one loop of bacteria and incubated for 1x24 hours at 37 ° C. Observed levels of turbidity.
b. **Testing MKC (Minimum Kill Concentration)**
   GNA Medium 10 ml put into a petri dish and then allowed to solidify and then stroked each incubation the MIC test results, 1x subsequently incubated for 24 hours at 37 ° C. KBM value indicated by the absence of microbial growth at the lowest concentration of samples (Mufid Khunafi, 2010).

Antibacterial activity test

Antibacterial activity test of ethanol extract of white pumpkin leaves (Lagenaria siceraria) performed by the agar diffusion method using a concentration of 0.5%, 1% and 2% in the positive control chloramphenicol then retrieved GNA medium 10 ml was poured into a petri dish to solidify. Then streaking bacteria in basic medium that has been solidified by using a swab bad. Paper discs that had been soaked in vials containing samples with concentrations each placed in a petri dish that already contains medium and microbial suspension. Incubated 1x24 hours at a temperature of 370C, then observed inhibition zone formed.

Result and Discussion

From the research that had been done on the ethanol extract of white pumpkin leaves (Lagenaria siceraria) against some pathogenic bacteria obtained as follows:

Table 3. Observations screening test (preliminary test) antibacterial ethanol extract of white pumpkin leaves (Lagenaria siceraria)

Sample	Microbe Test							
	EC	PA	SA	ST	VB	SM	BS	SE
Ethanol extract of white pumpkin leaves (Lagenaria siceraria)	+	-	+	-	-	+	-	+

\+ : No growth of bacteria
\- : No growth of bacteria.
EC: *Escherichia coli*
PA: *Pseudomonas aeroginosa*
SA: *Staphylococcus aureus*
ST: *Salmonella thypi*
VB: *Vibrio sp*
SM: *Streptococcus mutans*
BS: *Bacillus subtilis*
SE:*Staphylococcus epidermidis*

Table 4. Diameter of Inhibition ethanol extract of white pumpkin leaves (Lagenaria siceraria) against bacterial pathogens

Bacteria	Replication	Concentration			Positive Control (Kloramfenikol)
		0,5 %	1 %	2 %	
		Diameter (cm) inhibition zone of ethanol extract of white pumpkin leaves (*Lagenaria siceraria*)			
		0,5 %	1 %	2 %	
Staphylococcus aureus	I	0,8	0,9	1	2,8
	II	0,7	0,8	0,9	2,8
	III	0,8	0,7	0,9	2,8
	Average	1,76	1,93	2,2	2,8
Escherichia coli	I	0,8	1,4	1,7	2
	II	0,7	1,4	1,8	2,1
	III	0,7	1,3	1,8	2
	Average	1,73	3,23	4,1	4,76
Staphylococcus epidermidis	I	0,7	1	1,1	2,5
	II	0,8	0,9	1	2,6
	III	0,8	0,9	1	2,5
	Average	1,76	2,2	2,43	5,93
Streptococcus mutans	I	0,9	1,6	1,5	3,5
	II	0,8	1,5	1,7	3,5
	III	0,8	1,5	1,6	3,2
	Average	1,96	3,6	3,73	8,06

Discussion

The sample used in the study was white pumpkin leaves (Lagenaria siceraria), which white pumpkin leaves (Lagenaria siceraria) is extracted for obtaining the active substance in the sample. According harmone in wawolumaya 2012, white pumpkin leaves contains tannin which one tannin which serves as an antiseptic is saponins which are secondary metabolites that can form foam, so hopefully with a polar solvent is 96% ethanol can attract tannin especially saponins on the sample. The ethanol extract of white pumpkin (Lagenaria siceraria) further screened its anti-bacteria activity using jelly diffusion method, this test is a preliminary test to determine the antibacterial activity of a sample. The results obtained are used for further testing.

Preliminary test carried out by way of a screening test against 8 bacterial pathogens, namely: Escherichia coli, Vibrio sp, Streptococcus mutans, Stapylacoccus aureus, Salmonella thypi, Pseudomonas aeroginosa, Bacillus subtilis and Staphylococcus epidermidis with medium NA which then observed the presence or absence of bacterial growth at concentrations of 0 , 5% and 1%. Results obtained from the observation that there are four bacteria that does not hinder that Vibrio sp, thypi Salmonella, Bacillus subtilis and Pseudomonas aeroginosa. This could be due to the low concentration of the extract used and is unable to inhibit pathogenic bacteria that and it can be caused by gram-negative bacteria that has Bacteriocins-like inhibitor aubstances (BLIS) is a bacterium that serves deter foreign substance from other organisms in one strain, between species or from the environment into the cell. Where, concentration used was 0.5%. While the bacteria can be inhibited by the extract of white pumpkin leaves are Escherichia coli, Streptococcus mutans, Stapylacoccus aureus and epidermidis Staphylacoccus at the same concentration of 0.5%.

Based on the results of screening test, then test the MIC (Minimum Inhibitory Concentration) using the four bacterium and they are Escherichia coli, Streptococcus mutans, Stapylacoccus aureus and epidermidis Staphylacoccus. This test was conducted to determine the specific inhibitory concentration produced by the ethanol extract of white pumpkin leaves against pathogens after a screening test or preliminary test. In this MIC test for the presence of bacteria, it is marked by the turbidity in each tube. In this test the samples used 3 concentrations of ethanol extract of white pumpkin leaves (Lagenaria siceraria) (% w / v) of 0.5%; 1%; 2%. In this study, the use of chloramphenicol is as a positive control.

Where, chloramphenicol is a bacteriostatic antibiotic that has activity. Bacteriostatic compounds often inhibit protein synthesis or binding of ribosomes, at high doses are bacteriostatic. Bacteriostatic compounds effect by inhibiting the growth of cells in the logarithmic phase so that the number of living cells decreased. Chloramphenicol give effect to the way it reacts to the 50S sub-unit of the ribosome and inhibit peptidyl transferase enzyme activity. This enzyme serves to form a peptide bond between amino acids new still attached to tRNA with the last amino acid that is growing. As a result, the bacterial protein synthesis would stop immediately (Pratiwi, 2008). Chloramphenicol is effective against aerobic gram-positive bacteria, including Salmonella thypi. Chloramphenicol is an antibiotic which has a broad spectrum of work. So that

chloramphenicol is used as a positive control.

The results obtained in the MIC test is at a concentration of 0.5%, the ethanol extract of white pumpkin leaves could inhibit the growth of bacteria Escherichia coli, Streptococcus mutans, Stapylacoccus aureus, and Staphylacoccus epidermidi. Further testing is testing a minimum kill concentration (MBC) with the scratch method using GNA medium to each sample concentration. This test was done to determine the minimum kill concentration of ethanol extract of white pumpkin leaves against bacterial pathogens used. The results obtained in the KBM test after incubated for 1 x 24 hours was obtained at a concentration of 2%, the sample is capable of killing bacteria Escherichia coli, Streptococcus mutans, Stapylacoccus aureus and epidermidis Staphylacoccus.

Based on the screening results, further testing antibacterial inhibition is done by jelly diffusion method (medium GNA). The results obtained in the test of inhibition is in bacteria Escherichia coli with a diameter of obstacles each concentration of 0.5%; 1% and 2%, ie 1.73 cm; 3.23 cm; and 4.1 cm. At the bacterium Streptococcus mutans in diameter on inhibition respectively concentration of 0.5%; 1% and 2%, ie 1,96 cm; 3.6 cm; 3.73 cm. while at Stapylacoccus aureus bacteria with a concentration of 0.5%; 1% and 2%, ie 1.76 cm; 1.93 cm, and 2.2 cm. the bacteria staphylococcus epidermidis with a concentration of 0.5%; 1%; 2% is obtained diameter of 1.76 cm; 2.2 cm; and 2.43 cm. Referring to the general standards issued by the Ministry of Health (1988) in Anand (2007) stated that the microbes otherwise sensitive to antimicrobial plant origin when a diameter of power constraints 1.2 cm - 2.4 cm or equal to 12 mm - 24 mm. The results showed that the ethanol extract of white pumpkin leaves (Lagenaria siceraria) with a diameter of inhibition produced in accordance with the standards prescribed by the Ministry of Health, namely a diameter of 1.2 cm - 2.4 cm or equal to 12 mm - 24 mm.

Meanwhile, according to Suriawiria in Rahmawati (2006) measurement of the strength of the antibiotic based methods David-Stouts, said if the clear zone diameter of 5 mm or equal to 0.5 cm showed weak antibacterial activity, a diameter of 5-10 mm, equivalent to 0.5-1 cm showed moderate antibacterial activity, diameter 10-20 mm, equivalent to 1-2 cm showed strong antibacterial activity and a diameter of 20 mm or equal to 2 cm showed very strong antibacterial activity. Thus, based on these descriptions ethanol extract of white pumpkin leaves at a concentration of 0.5% including strong antibacterial category on four bacterial pathogens used (Escherichia coli, Streptococcus mutans, Stapylacoccus aureus and epidermidis Staphylacoccus) due to inhibition zone diameters obtained ranged 10- 20 mm or the equivalent of 1-2 cm, while at a concentration of 1% included into the strong category in Staphylacoccus aureus bacteria for inhibitory zone diameters obtained range from 10-20 mm, equivalent to 1-2 cm and included a very strong antibacterial category in bacteria Escherichia coli, Streptococcus mutans, and Staphylacoccus epidermidis because the diameter of inhibition zone were obtained ranging from 20 mm or equal to 2 cm. While at a concentration of 2% ethanol extract of leaves of pumpkin white category antibacterial very strong against pathogens used (Escherichia coli, Streptococcus mutans, Stapylacoccus aureus, and Staphylacoccus epidermidis) since the diameter of inhibition zone were obtained ranging from 20 mm or equal to 2 cm. It concluded that : (1) The activities white pumpkin leaves (Lagenaria siceraria) could inhibit some pathogenic bacteria of 8 bacteria used include Escherichia coli, Streptococcus mutans, Stapylacoccus aureus, and Staphylacoccus epidermidis, while the white pumpkin leaves are not able to inhibit the growth of bacteria Vibrio sp, Salmonella thypi, Bacillus subtilis and Pseudomonas aeroginosa. Where empirically white pumpkin leaves are used as traditional medicine, this white pumpkin leaves are commonly used as a remedy for skin rashes and as a drug that helps the digestive process; (2) Leaves white pumpkin (Lagenaria siceraria) could inhibit some pathogenic bacteria (Escherichia coli, Streptococcus mutans, Stapylacoccus aureus and epidermidis Staphylacoccus) at a concentration of 0.5% with the optimum concentration of 2%.

From the results obtained, the white pumpkin leaves inhibit bacterial Stapylacoccus Staphylacoccus epidermidis and aureus. Where, it is known that both of these bacteria can cause irritation to the skin such as itching, acne, boils and dandruff. This fits the empirical data obtained that white pumpkin leaves used as a traditional medicine, especially as a remedy for itchy skin, the bacterium Escherichia coli, white pumpkin leaves proved effective in inhibiting the bacteria Escherichia coli where it is known that Escherichia coli bacteria can cause diarrhea, sepsis or depression, shock and the like. Other bacterial leaf on white pumpkin is able to inhibit the growth of bacteria Streptococcus mutans, which these bacteria can cause dental caries. However, there has been no empirical data obtained on the use of white pumpkin leaves against a disease caused by the bacterium Streptococcus mutans. Inhibition regional observed visually that the inhibition increased with increasing concentrations of ethanol extract of white pumpkin leaves (Lagenaria siceraria).

Ethanol extract of white pumpkin leaves (Lagenaria siceraria) showed inhibitory effect on several pathogenic bacteria, namely Streptococcus mutans, Stapylacoccus aureus, Escherichia coli and Staphylacoccus epidermidis with the results of analysis of variance as well as the F table area inhibitory ethanol extract of white pumpkin leaves against some pathogenic bacteria (Streptococcus mutans, Stapylacoccus aureus and epidermidis Staphylacoccus) show significant gains or has the smallest difference where F count> F table at the level of 95%. In the test results Honestly Significant Difference (HSD), showed that the sample concentration of 0.5%, 1% and

2% were significantly different to the positive control, so it has not obtained the optimum concentration equivalent to the positive control.

Conclusion

Based on the research that had been done could be concluded that: (1) The ethanol extract of white pumpkin leaves (Lagenaria Siceraria) has antibacterial activity against bacteria Staphylacoccus aureus, staphylacoccus mutans, Staphylococcus epidermidis and E. Coli. (2) The ethanol extract of white pumpkin leaves (Lagenaria Siceraria) can inhibit bacteria staphylacoccus aureus, staphylacoccus mutans, staphylococcus epidermidis and E. coli at a concentration of 0.5% and kill bacteria at a concentration of 2%.

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